

Long term transcriptional and behavioral effects in mice developmentally exposed to a mixture of endocrine disruptors associated with delayed human neurodevelopment.

Anastasia Repouskou^{1‡}, Anastasia-Konstantina Papadopoulou^{1,2‡}, Emily Panagiotidou^{1,2}, Panagiotis Trichas², Christian Lindh³, Åke Bergman⁴, Chris Gennings⁵, Carl-Gustaf Bornehag^{5,6}, Joëlle Rüegg⁷, Efthymia Kitraki^{1#*}, Antonios Stamatakis^{2#*}

[‡] equal contribution

equal contribution

¹Basic Sciences lab, Faculty of Dentistry, School of Health Sciences, National and Kapodistrian University of Athens (NKUA), Athens, Greece

²Biology-Biochemistry lab, Faculty of Nursing, School of Health Sciences, NKUA, Athens, Greece

³Division of Occupational and Environmental Medicine, Department of Laboratory Medicine, Lund University, Lund, Sweden

⁴Department of Environmental Science, Stockholm University, SE-106 91 Stockholm, Sweden

⁵Icahn School of Medicine at Mount Sinai, New York, NY, USA

⁶Karlstad University, Karlstad, Sweden

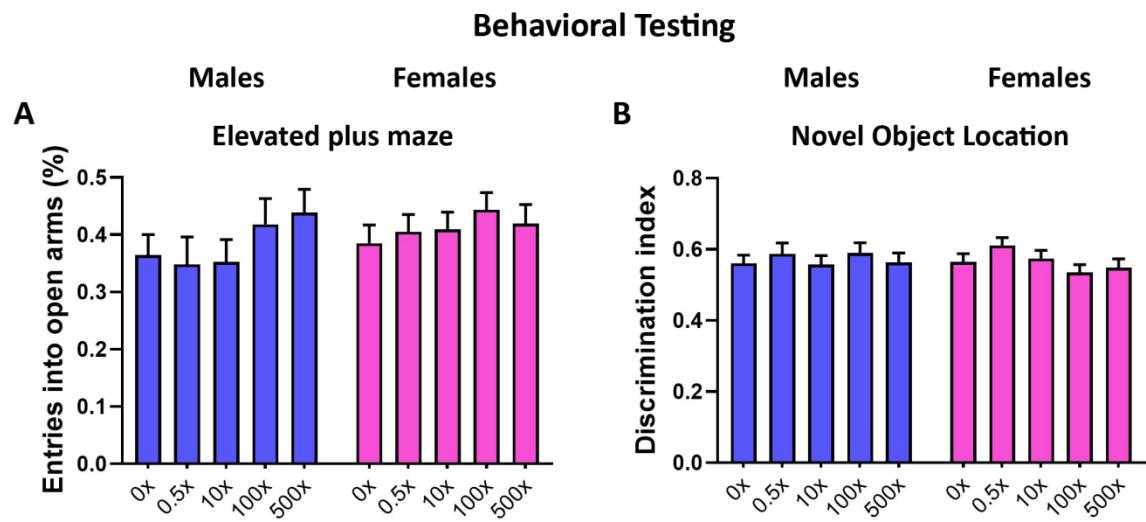
⁷Uppsala University, Evolutionary Biology Centre, Department of Organismal Biology, Norbyvägen 18A, 752 36 Uppsala

*Corresponding authors:

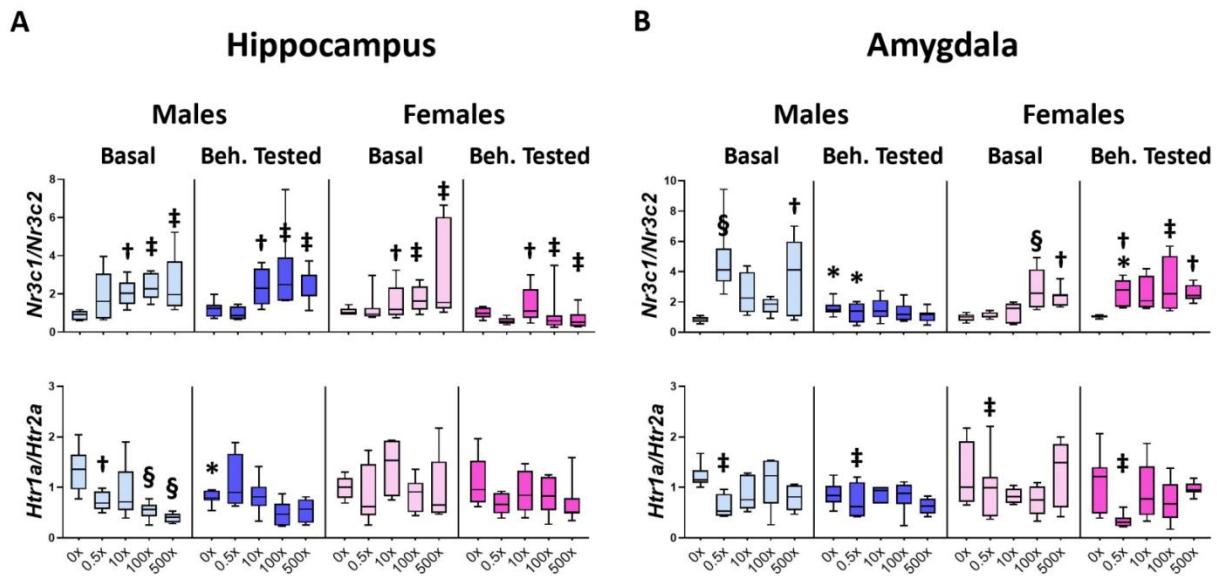
Antonios Stamatakis Faculty of Nursing, School of Health Sciences, National and Kapodistrian University of Athens, (NKUA), Papadiamantopoulou 123, 11527 Athens, Greece.
astam@nurs.uoa.gr

Efthymia Kitraki Faculty of Dentistry, School of Health Sciences, NKUA, Thivon 2 Goudi, 11527 Athens, Greece. ekitraki@dent.uoa.gr

SUPPLEMENTAL FIGURES AND TABLES

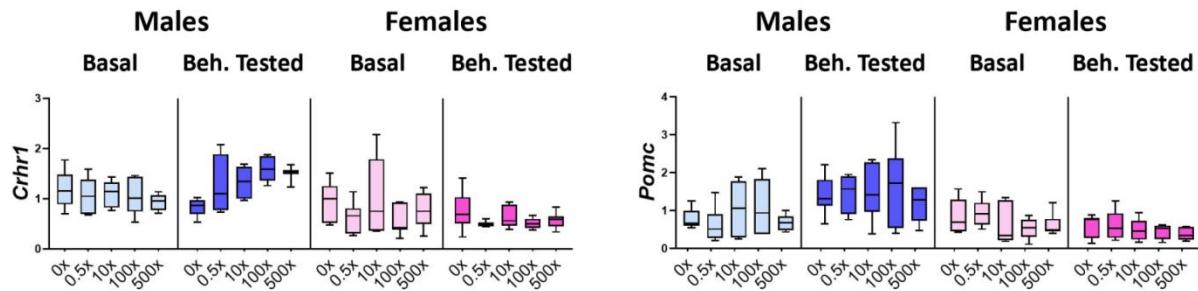


Supplementary Figure S1. Behavioral responses of adult male (blue bars) and female (magenta bars) mice *in utero* exposed to 0.5x, 10x, 100x and 500x SELMA mothers' levels of mixture N1 or the vehicle (0x) in the Elevated plus maze (A) and the Novel object location (B) tests. Bars represent the estimated marginal means \pm SEM. Significance was accepted for $P < 0.05$. No significant effects of mixture N1 exposure were detected.



Supplementary Figure S2. Effect of mixture N1 on the *Nr3c1/Nr3c2* and *Htr1a/Htr2a* expression level ratios in (A) the hippocampus and (B) the amygdala of adult male (blue bars) and female (magenta bars) mice *in utero* exposed to 0.5x, 10x, 100x and 500x SELMA mothers' levels of mixture N1 or the vehicle (0x). Light colored bars show gene expression ratios under basal conditions (Basal) and darkly colored bars the gene expression ratios of matched siblings that underwent the behavioral tests (Beh. Tested). Expression levels of indicated genes were evaluated by qRT-PCR and normalized to *b-actin*. Box plots encompassing values from the 25th to 75th percentile of the data. The horizontal line in the box shows the median value, whereas the horizontal lines above and below the box show the maximal and minimal values, respectively. Significance was accepted for $P < 0.05$. Significance is shown for the effect of mixture vs. the respective (Basal or Beh. Tested) control. + $0.01 < P < 0.05$, # $0.001 < P < 0.01$, § $P < 0.001$ as well as for the effect of Beh. Testing vs. the respective Basal group * $P < 0.05$. Detailed statistics are provided in the Results section as well as in Supplemental tables S3, S3.1, S4 & S4.1.

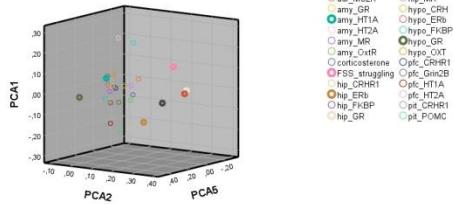
Pituitary



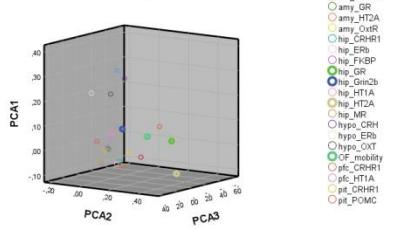
Supplementary Figure S3. Effect of mixture N1 on pituitary gene expression of adult male (blue bars) and female (magenta bars) mice *in utero* exposed to 0.5x, 10x, 100x and 500x SELMA mothers' levels of mixture N1 or the vehicle (0x). Light colored bars show gene expression under basal conditions (Basal) and darkly colored bars the gene expression of matched siblings that underwent the behavioral tests (Beh. Tested). Expression levels of indicated genes were evaluated by qRT-PCR and normalized to *b-actin*. Box plots encompassing values from the 25th to 75th percentile of the data. The horizontal line in the box shows the median value, whereas the horizontal lines above and below the box show the maximal and minimal values, respectively. Significance was accepted for $P < 0.05$. No significant effects of mixture N1 exposure were detected.

Males

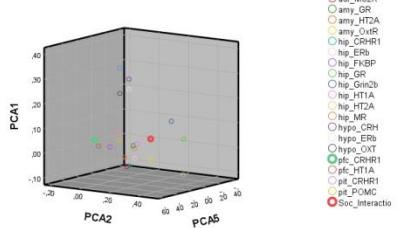
A Struggling in FSS



B Mobility in OF

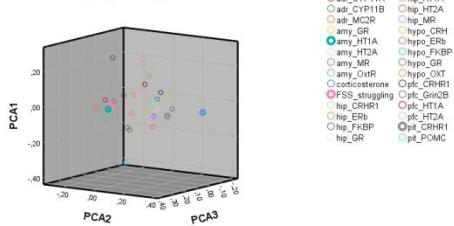


C Social Interaction



Females

D Struggling in FSS



Supplementary Figure S4. Principal Component Analysis plots. Component plots in rotated space depicting Component Score Coefficients for levels of gene expression and behavioral scores for (A) struggling during Forced Swim Stress (FSS) of males, (B) mobility in open field (OF) of males, (C) social interaction of males and (D) struggling during Forced Swim Stress (FSS) of females. In each panel, the respective behavioral score and the genes clustered along with it are shown in bold circles.

Table S1. W values of the GLN and the respective Bonferroni post hoc comparisons between the DMSO-treated and the Mixture N1-treated groups are provided for all behaviors analyzed in this study (N/A: post hoc was not applicable). Significance was set for P < 0.05.

Elevated plus maze MALES	GLN W/P values	0.5x	10x	100x	500x
Entries into closed arms	5.811/0.214	N/A	N/A	N/A	N/A
Entries into open arms	5.241/0.263	N/A	N/A	N/A	N/A
% entries into open arms	2.239/0.692	N/A	N/A	N/A	N/A
Time in closed arms	2.125/0.713	N/A	N/A	N/A	N/A
Time in open arms	3.055/0.549	N/A	N/A	N/A	N/A
% time in open arms	2.324/0.676	N/A	N/A	N/A	N/A
Elevated plus maze FEMALES	GLN W/P values	0.5x	10x	100x	500x
Entries into closed arms	5.811/0.214	N/A	N/A	N/A	N/A
Entries into open arms	5.241/0.263	N/A	N/A	N/A	N/A
% entries into open arms	2.239/0.692	N/A	N/A	N/A	N/A
Time in closed arms	2.125/0.713	N/A	N/A	N/A	N/A
Time in open arms	3.055/0.549	N/A	N/A	N/A	N/A
% time in open arms	2.324/0.676	N/A	N/A	N/A	N/A

Social Interaction MALES	GLN W/P values	0.5x	10x	100x	500x
Discrimination Index	28.576/<0.001	0.002	1.000	1.000	0.031
Social Interaction FEMALES	GLN W/P values	0.5x	10x	100x	500x
Discrimination Index	1.861/0.761	N/A	N/A	N/A	N/A

Table S1. (continued)

Open Field MALES	GLN W/P values	0.5x	10x	100x	500x
Total distance moved	20.485/<0.001	1.000	0.010	0.008	0.003
Time in center	2.976/0.562	N/A	N/A	N/A	N/A
Frequency in center	7.681/0.104	N/A	N/A	N/A	N/A
Free rearings	1.260/0.868	N/A	N/A	N/A	N/A
Rearings against walls	1.256/0.869	N/A	N/A	N/A	N/A
Open Field FEMALES	GLN W/P values	0.5x	10x	100x	500x
Total distance moved	10.954/0.027	N/A	N/A	N/A	N/A
Time in center	2.976/0.562	N/A	N/A	N/A	N/A
Frequency in center	7.681/0.104	N/A	N/A	N/A	N/A
Free rearings	1.260/0.868	N/A	N/A	N/A	N/A
Rearings against walls	1.256/0.869	N/A	N/A	N/A	N/A
Novel Object Location MALES	GLN W/P values	0.5x	10x	100x	500x
Memory Index	4.172/0.383	N/A	N/A	N/A	N/A
Novel Object Location FEMALES	GLN W/P values	0.5x	10x	100x	500x
Memory Index	4.172/0.383	N/A	N/A	N/A	N/A
Forced Swim Stress MALES	GLN W/P values	0.5x	10x	100x	500x
Struggling	21.321/<0.001	0.686	0.009	0.001	0.003
Floating	2.282/0.684	N/A	N/A	N/A	N/A
Forced Swim Stress FEMALES	GLN W/P values	0.5x	10x	100x	500x
Struggling	21.321/<0.001	0.686	0.009	0.001	0.003
Floating	2.282/0.684	N/A	N/A	N/A	N/A

Table S2. Hypothalamus. F and P values of the ANOVAs for the effect of Mixture N1 and P values of the Dunnett's post hoc comparisons between the DMSO-treated and the Mixture N1-treated groups are provided for all genes analyzed in this study (B: Basal animals; BT: Behaviorally tested). N/A: post hoc was not applicable). Significance was set for P < 0.05.

Hypothalamus MALES	F (B / BT) P (B / BT)	Post hoc 0.5 x (B / BT)	Post hoc 10x (B / BT)	Post hoc 100x (B / BT)	Post hoc 500x(B / BT)
<i>Crh</i>	4.645/4.645 0.003/0.003	0.51 / 0.51	0.98 / 0.98	0.011 / 0.011	0.66 / -0.66
<i>Nr3c1</i>	3.248/3.125 0.031/0.033	0.44 / 0.59	0.92 / 0.06	0.040 / 0.16	0.46 / 0.010
<i>Fkbp5</i>	1.695/10.774 0.184/ <0.001	N/A / 0.27	N/A / 0.19	N/A / <0.001	N/A / <0.001
<i>Oxt</i>	9.720/9.720 <0.001 / <0.001	0.99 / 0.99	0.81 / 0.81	<0.001 / <0.001	0.001 / 0.001
<i>Esr2</i>	9.066/9.066 <0.001 / <0.001	0.60 / 0.60	0.96 / 0.96	<0.001 / <0.001	0.09/ 0.09
Hypothalamus FEMALES	F (B / BT) P (B / BT)	Post hoc 0.5 x (B / BT)	Post hoc 10x (B / BT)	Post hoc 100x (B / BT)	Post hoc 500x(B / BT)
<i>Crh</i>	1.530/1.530 0.209/0.209	N/A	N/A	N/A	N/A
<i>Nr3c1</i>	1.949/0.514 0.134/0.726	N/A	N/A	N/A	N/A
<i>Fkbp5</i>	0.262/1.531 0.897/0.221	N/A	N/A	N/A	N/A
<i>Oxt</i>	0.637/0.637 0.638/0.638	N/A	N/A	N/A	N/A
<i>Esr2</i>	1.071/1.071 0.382/0.382	N/A	N/A	N/A	N/A

Table S2.1. Hypothalamus. F and P values of the ANOVAs for the effect of Mixture N1 and P values of the independent samples t- tests between the Basal and Behaviorally tested offspring per treatment group. F and P values refer to the triple interaction (treatment x behavioral testing x sex). N/A: the comparisons were not applicable. Significance was set for P < 0.05.

Hypothalamus MALES	F / P values	DMSO P values	0.5x P values	10x P values	100x P values	500x P values
<i>Crh</i>	1.766/0.143	N/A	N/A	N/A	N/A	N/A
<i>Nr3c1</i>	2.897/0.026	0.089	0.089	0.099	0.011	0.69
<i>Fkbp5</i>	2.948/0.024	0.003	0.003	0.806	0.177	0.109
<i>Oxt</i>	0.145/0.965	N/A	N/A	N/A	N/A	N/A
<i>Esr2</i>	0.993/0.416	N/A	N/A	N/A	N/A	N/A
Hypothalamus FEMALES	F / P values	DMSO P values	0.5x P values	10x P values	100x P values	500x P values
<i>Crh</i>	1.766/0.143	N/A	N/A	N/A	N/A	N/A
<i>Nr3c1</i>	2.897/0.026	0.194	0.221	0.581	0.069	0.634
<i>Fkbp5</i>	2.948/0.024	0.809	0.523	0.114	0.003	0.876
<i>Oxt</i>	0.145/0.965	N/A	N/A	N/A	N/A	N/A
<i>Esr2</i>	0.993/0.416	N/A	N/A	N/A	N/A	N/A

Table S3. Hippocampus. F and P values of the ANOVAs for the effect of Mixture N1 and P values of the Dunnett's post hoc comparisons between the DMSO-treated and the Mixture N1-treated groups are provided for all genes analyzed in this study (B: Basal animals; BT: Behaviorally tested). N/A: post hoc was not applicable). Significance was set for P < 0.05.

Hippocampus MALES	F (B / BT) P (B / BT)	Post hoc 0.5 x (B / BT)	Post hoc 10x (B / BT)	Post hoc 100x (B / BT)	Post hoc 500x(B / BT)
<i>Nr3c1</i>	5.563/5.563 0.001 / 0.001	0.75 / 0.75	0.002 / 0.002	0.001 / 0.001	0.20 / 0.20
<i>Nr3c2</i>	3.771/3.246 0.015/0.028	0.48 / 0.20	0.021 / 0.99	0.038 / 0.42	0.012 / 0.48
<i>Nr3c1 / Nr3c2</i>	5.424 / 5.424 0.001 / 0.001	0.961 / 0.961	0.034 / 0.034	0.006 / 0.006	0.002 / 0.002
<i>Fkbp5</i>	1.801 0.134	N/A	N/A	N/A	N/A
<i>Esr2</i>	0.871 0.484	N/A	N/A	N/A	N/A
<i>Crhr1</i>	9.125 /9.125 <0.001/<0.001	0.97 / 0.97	0.72 / 0.72	0.028 / 0.028	0.001 / 0.001
<i>Htr1a</i>	11.628 /9.147 <0.001/<0.001	0.002 / 1.00	0.001 / 0.25	0.001 / 0.008	<0.001 / 0.024
<i>Htr2a</i>	1.413/1.413 0.242/0.242	N/A	N/A	N/A	N/A
<i>Htr1a / Htr2a</i>	7.361/3.176 <0.001/0.031	0.013 / 0.412	0.109 / 0.999	0.001 / 0.221	<0.001 / 0.385
<i>Grin2b</i>	3.246 0.015	0.40 / 0.40	0.40 / 0.40	0.08 / 0.08	0.85 / 0.85
Hippocampus FEMALES	F (B / BT) P (B / BT)	Post hoc 0.5 x (B / BT)	Post hoc 10x (B / BT)	Post hoc 100x (B / BT)	Post hoc 500x(B / BT)
<i>Nr3c1</i>	0.233/0.233 0.919/0.919	N/A	N/A	N/A	N/A
<i>Nr3c2</i>	2.124 / 3.756 0.108 / 0.014	N/A / 0.12	N/A / 0.40	N/A / 0.31	N/A / 0.97
<i>Nr3c1 / Nr3c2</i>	5.424 / 5.424 0.001 / 0.001	0.961 / 0.961	0.034 / 0.034	0.006 / 0.006	0.002 / 0.002
<i>Fkbp5</i>	1.801 0.134	N/A	N/A	N/A	N/A
<i>Esr2</i>	0.871 0.484	N/A	N/A	N/A	N/A
<i>Crhr1</i>	2.080/2.080 0.095/0.095	N/A	N/A	N/A	N/A
<i>Htr1a</i>	0.792/1.180 0.542/0.341	N/A	N/A	N/A	N/A
<i>Htr2a</i>	3.574/3.574	0.09 / 0.09	0.86 / 0.86	0.66 / 0.66	0.07 / 0.07

	0.011/0.011				
<i>Htr1a / Htr2a</i>	1.243/1.448 0.317/0.243	N/A	N/A	N/A	N/A
<i>Grin2b</i>	3.246 0.015	0.40 / 0.40	0.40 / 0.40	0.08 / 0.08	0.85 / 0.85

Table S3.1. Hippocampus. F and P values of the ANOVAs for the effect of Mixture N1 and P values of the independent samples t- tests between the Basal and Behaviorally tested offspring per treatment group. F and P values refer to the triple interaction (treatment x behavioral testing x sex). N/A: the comparisons were not applicable. Significance was set for P < 0.05.

Hippocampus MALES	F / P values	DMSO P values	0.5x P values	10x P values	100x P values	500x P values
<i>Nr3c1</i>	1.722/0.151	N/A	N/A	N/A	N/A	N/A
<i>Nr3c2</i>	2.998/0.022	0.763	0.119	0.025	0.512	0.313
<i>Fkbp5</i>	0.680/0.607	N/A	N/A	N/A	N/A	N/A
<i>Esr2</i>	2.301/0.064	N/A	N/A	N/A	N/A	N/A
<i>Crhr1</i>	2.388/0.056	N/A	N/A	N/A	N/A	N/A
<i>Htr1a</i>	3.295/0.014	0.081	0.058	0.042	0.049	0.180
<i>Htr2a</i>	0.495/0.740	N/A	N/A	N/A	N/A	N/A
<i>Htr1a / Htr2a</i>	2.517/0.046	0.019	0.175	0.837	0.648	0.148
<i>Grin2b</i>	1.108/0.357	N/A	N/A	N/A	N/A	N/A
Hippocampus FEMALES	F / P values	DMSO P values	0.5x P values	10x P values	100x P values	500x P values
<i>Nr3c1</i>	1.722/0.151	N/A	N/A	N/A	N/A	N/A
<i>Nr3c2</i>	2.998/0.022	0.956	0.131	0.049	0.038	0.195
<i>Fkbp5</i>	0.680/0.607	N/A	N/A	N/A	N/A	N/A
<i>Esr2</i>	2.301/0.064	N/A	N/A	N/A	N/A	N/A
<i>Crhr1</i>	2.388/0.056	N/A	N/A	N/A	N/A	N/A
<i>Htr1a</i>	3.295/0.014	0.693	0.872	0.135	0.08	0.127
<i>Htr2a</i>	0.495/0.740	N/A	N/A	N/A	N/A	N/A
<i>Htr1a / Htr2a</i>	2.517/0.046	0.639	0.487	0.118	0.953	0.358
<i>Grin2b</i>	1.108/0.357	N/A	N/A	N/A	N/A	N/A

Table S4. Amygdala. F and P values of the ANOVAs for the effect of Mixture N1 and P values of the Dunnett's post hoc comparisons between the DMSO-treated and the Mixture N1-treated groups are provided for all genes analyzed in this study (B: Basal animals; BT: Behaviorally tested). N/A: post hoc was not applicable). Significance was set for P < 0.05.

Amygdala MALES	F (B / BT) P (B / BT)	Post hoc 0.5 x (B / BT)	Post hoc 10x (B / BT)	Post hoc 100x (B / BT)	Post hoc 500x(B / BT)
<i>Nr3c1</i>	2.853/2.321 0.044 / 0.085	0.09 / 0.06	0.99 / 0.38	0.99 / 0.11	0.06 / 0.08
<i>Nr3c2</i>	17.575 / 0.255 <0.001 /0.904	<0.001 /N/A	<0.001 / N/A	<0.001 / N/A	<0.001 / N/A
<i>Nr3c1 / Nr3c2</i>	5.404/0.701 0.003 / 0.599	0.001 / N/A	0.215 / N/A	0.713/ N/A	0.024 / N/A
<i>Htr1a</i>	3.503/2.023 0.020 /0.122	0.037 / N/A	0.043 / N/A	1.00 / N/A	0.39 / N/A
<i>Htr2a</i>	2.154/4.922 0.104 / 0.005	N/A / 1.00	N/A / 0.08	N/A / 0.84	N/A / 0.16
<i>Htr1a / Htr2a</i>	5.927 /5.927 0.017 / 0.017	0.002 / 0.002	0.136 / 0.136	0.101/0.101	0.342 / 0.342
<i>Oxtr</i>	0.603/0.603 0.662/ 0.662	N/A	N/A	N/A	N/A
Amygdala FEMALES	F (B / BT) P (B / BT)	Post hoc 0.5 x (B / BT)	Post hoc 10x (B / BT)	Post hoc 100x (B / BT)	Post hoc 500x(B / BT)
<i>Nr3c1</i>	2.995/10.933 0.039 /< 0.001	1.00 / <0.001	1.00 / 0.47	0.17 / 0.049	0.08 / <0.001
<i>Nr3c2</i>	0.968/1.033 0.443/0.412	N/A	N/A	N/A	N/A
<i>Nr3c1 / Nr3c2</i>	7.600/4.612 <0.001 / 0.007	0.991 / 0.014	0.882 / 0.066	0.001 / 0.007	0.016 / 0.015
<i>Htr1a</i>	2.572 /8.933 0.064/< 0.001	N/A / 0.54	N/A / 0.96	N/A / 0.95	N/A / 0.001
<i>Htr2a</i>	0.563/7.895 0.692 /< 0.001	N/A / 0.001	N/A / 0.83	N/A / 0.58	N/A / 0.001
<i>Htr1a / Htr2a</i>	5.927 /5.927 0.017 / 0.017	0.002 / 0.002	0.136 / 0.136	0.101/0.101	0.342 / 0.342
<i>Oxtr</i>	6.279/6.279 <0.001 / <0.001	1.00/ 1.00	0.58 / 0.58	0.99 / 0.99	<0.001 / < 0.001

Table S4.1. Amygdala. F and P values of the ANOVAs for the effect of Mixture N1 and P values of the independent samples t- tests between the Basal and Behaviorally tested offspring per treatment group. F and P values refer to the triple interaction (treatment x behavioral testing x sex). N/A: the comparisons were not applicable. Significance was set for P < 0.05.

Amygdala MALES	F / P values	DMSO P values	0.5x P values	10x P values	100x P values	500x P values
<i>Nr3c1</i>	6.840/<0.001	0.128	0.002	0.966	0.478	0.095
<i>Nr3c2</i>	2.501/0.047	0.026	0.027	0.036	0.183	0.064
<i>Nr3c1/Nr3c2</i>	6.063/<0.001	0.004	0.029	0.13	0.215	0.091
<i>Htr1a</i>	2.976/0.023	0.117	0.108	0.404	0.223	0.236
<i>Htr2a</i>	3.407/0.012	0.057	0.448	0.153	0.513	0.664
<i>Oxtr</i>	1.795/0.136	N/A	N/A	N/A	N/A	N/A
Amygdala FEMALES	F /P values	DMSO P values	0.5x P values	10x P values	100x P values	500x P values
<i>Nr3c1</i>	6.840/<0.001	0.698	0.005	0.114	0.529	0.051
<i>Nr3c2</i>	2.501/0.047	0.961	0.534	0.536	0.308	0.150
<i>Nr3c1/Nr3c2</i>	6.063/<0.001	0.5	0.008	0.1	0.867	0.432
<i>Htr1a</i>	2.976/0.023	0.145	0.009	0.343	0.877	0.016
<i>Htr2a</i>	3.407/0.012	0.313	0.053	0.062	0.845	0.002
<i>Oxtr</i>	1.795/0.136	N/A	N/A	N/A	N/A	N/A

Table S5. Prefrontal cortex. F and P values of the ANOVAs for the effect of Mixture N1 and P values of the Dunnett's post hoc comparisons between the DMSO-treated and the Mixture N1-treated groups are provided for all genes analyzed in this study (B: Basal animals; BT: Behaviorally tested). N/A: post hoc was not applicable). Significance was set for P < 0.05

Prefrontal MALES	F (B / BT) P (B / BT)	Post hoc 0.5 x (B / BT)	Post hoc 10x (B / BT)	Post hoc 100x (B / BT)	Post hoc 500x(B / BT)
<i>Crhr1</i>	3.585/3.585 0.012/ 0.012	0.13 / 0.13	0.023 / 0.023	0.011 / 0.011	0.85 / 0.85
<i>Grin2b</i>	14.384 /1.523 <0.001/0.208	<0.001/ N/A	<0.001/ N/A	<0.001/ N/A	<0.001/ N/A
<i>Htr1a</i>	2.141/2.141 0.092/0.092	N/A	N/A	N/A	N/A
<i>Htr2a</i>	6.550/0.495 <0.001/0.739	<0.001/ N/A	0.001/ N/A	<0.001/ N/A	0.003 / N/A
<i>Htr1a/ Htr2a</i>	1.140 0.342	N/A	N/A	N/A	N/A
Prefrontal FEMALES	F (B / BT) P (B / BT)	Post hoc 0.5 x (B / BT)	Post hoc 10x (B / BT)	Post hoc 100x (B / BT)	Post hoc 500x(B / BT)
<i>Crhr1</i>	2.941/2.941 0.028/0.028	1.00 / 1.00	0.07 / 0.07	0.94 / 0.94	0.07 / 0.07
<i>Grin2b</i>	14.384 /1.523 <0.001/0.208	<0.001/ N/A	<0.001/ N/A	<0.001/ N/A	<0.001/ N/A
<i>Htr1a</i>	2.538/2.538 0.049/0.049	0.12 / 0.12	1.00 / 1.00	0.16 / 0.16	1.00 / 1.00
<i>Htr2a</i>	6.550/0.495 <0.001/0.739	<0.001 / N/A	0.001/ N/A	<0.001 / N/A	0.003 / N/A
<i>Htr1a/ Htr2a</i>	1.140 0.342	N/A	N/A	N/A	N/A

Table S5.1. Prefrontal Cortex. F and P values of the ANOVAs for the effect of Mixture N1 and P values of the independent samples t- tests between the Basal and Behaviorally tested offspring per treatment group. F and P values refer to the interaction (treatment x behavioral testing) since no triple interaction (treatment x behavioral testing x sex) was statistically significant for the analyzed genes in this brain area. N/A: the comparisons were not applicable. Significance was set for P < 0.05.

Prefrontal BOTH SEXES	F / P values	DMSO P values	0.5x P values	10x P values	100x P values	500x P values
<i>Crhr1</i>	0.819/0.516	N/A	N/A	N/A	N/A	N/A
<i>Grin2b</i>	2.961/0.023	0.741	0.008	0.002	0.001	0.003
<i>Htr1a</i>	0.720/0.580	N/A	N/A	N/A	N/A	N/A
<i>Htr2a</i>	2.583/0.042	0.494	< 0.001	0.001	0.001	0.002
<i>Htr1a/</i> <i>Htr2a</i>	1.259 / 0.291	N/A	N/A	N/A	N/A	N/A

Table S6. Adrenals. F and P values of the ANOVAs for the effect of Mixture N1 and P values of the Dunnett's post hoc comparisons between the DMSO-treated and the Mixture N1-treated groups are provided for all genes analyzed in this study (B: Basal animals; BT: Behaviorally tested). N/A: post hoc was not applicable). Significance was set for P < 0.05.

ADRENALS MALES	F (B / BT) P (B / BT)	Post hoc 0.5 x (B / BT)	Post hoc 10x (B / BT)	Post hoc 100x (B / BT)	Post hoc 500x(B / BT)
<i>Cyp11a1</i>	5.559/ 6.921 0.002/0.001	0.001 / 0.17	0.023 / 0.14	0.03 / <0.001	0.08 / 0.029
<i>Cyp11b1</i>	1.606/1.437 0.203/0.251	N/A	N/A	N/A	N/A
<i>Mc2r</i>	1.388/1.388 0.250	N/A	N/A	N/A	N/A
ADRENALS FEMALES	F (B / BT) P (B / BT)	Post hoc 0.5 x (B / BT)	Post hoc 10x (B / BT)	Post hoc 100x (B / BT)	Post hoc 500x(B / BT)
<i>Cyp11a1</i>	5.005/1.506 0.004 / 0.227	0.95 / N/A	0.019* / N/A *at low E2	1.00 / N/A	1.00 / N/A
<i>Cyp11b1</i>	1.922/3.268 0.139 / 0.026	N/A / 0.016	N/A / 0.12	N/A / 0.019	N/A / 0.14
<i>Mc2r</i>	9.035/9.035 <0.001/<0.001	0.048 / 0.048	0.040 / 0.040	<0.001/<0.001	<0.001/<0.001

Table S6.1. Adrenals. F and P values of the ANOVAs for the effect of Mixture N1 and P values of the independent samples t- tests between the Basal and Behaviorally tested offspring per treatment group. F and P values refer to the triple interaction (treatment x behavioral testing x sex). N/A: the comparisons were not applicable. Significance was set for P < 0.05.

Adrenals	F / P values	DMSO	0.5x	10x	100x	500x
MALES		P values	P values	P values	P values	P values
<i>Cyp11a1</i>	3.765/0.007	0.012	0.037	0.013	0.926	0.167
<i>Cyp11b1</i>	3.262/0.015	0.217	0.371	0.448	0.227	0.868
<i>Mc2r</i>	0.200/0.938	N/A	N/A	N/A	N/A	N/A
Adrenals	F / P values	DMSO	0.5x	10x	100x	500x
FEMALES		P values	P values	P values	P values	P values
<i>Cyp11a1</i>	3.765/0.007	0.498	0.102	0.032	0.159	0.029
<i>Cyp11b1</i>	3.262/0.015	0.236	0.026	0.072	0.257	0.137
<i>Mc2r</i>	0.200/0.938	N/A	N/A	N/A	N/A	N/A

Table S7. Daily exposure of pregnant mice ($\mu\text{g} / \text{Kg BW}$) through food to Mixture N1 components. x refers to the geometric mean of SELMA mothers' levels for the chemicals in Mixture N1.

Mixture N1 components	0.5x	10x	100x	500x
MEP	0.4349	86.975	869.747	4348.734
MBP	0.4430	88.595	885.952	4429.760
MBzP	0.1016	20.325	203.254	1016.271
MIDP/MPHP	0.0886	17.713	177.132	885.658
BPA	0.0075	1.500	14.999	74.994
TCP	0.0058	1.154	11.541	57.703
3-PBA	0.0016	0.329	3.294	16.469
p, p'-DDE	0.0131	2.623	26.226	131.129

Table S8. List of primers used in qRT-PCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	T annealing	Primer Bank ID / Reference
<i>Mc2r</i>	CATCTTGCAGAGAAAGATCCTA	CCTTGGCTTGTCACTAATGCT	58°C	Ref.1
<i>Cyp11a1</i>	AGGTCCCTCAATGAGATCCCTT	TCCCTGTAAATGGGCCATAC	58°C	9789921a1
<i>Cyp11b1</i>	GATAACAGATCCTGAGGGAGC	CCGGCAACGTCACAAACACA	59°C	Ref. 2
<i>Pomc</i>	TAGATGTGTGGAGCTGGTGC	CGTACTTCCGGGGTTTCA	58°C	This study
<i>Crhr1</i>	GGAACCTCATCTCGGCTTCA	GTTACGTGGAAGTAGTTGTAGGC	59°C	6681013a1
<i>Crh</i>	GAGGCATCCTGAGAGAAGTCC	GTTAGGGCGCTCTCTCTC	60°C	This study
<i>Nr3c1</i>	GGACCACCTCCAACTCTG	GCTGTCCTTCACTGCTCTT	60°C	This study
<i>Nr3c2</i>	GAAAGGGCTGGAGTCAGT	TGTTGGAGTAGCACCGGAA	60°C	17384009a1
<i>Fkbp5</i>	TGAGGGCACCACTAACATGG	CAACATCCCTTGAGTGGACAT	60°C	Ref. 3
<i>Esr2</i>	ACACCTTGCTGTAAACAGAGA	GCAGAAGTGAGCATCCCTCTT	58°C	46877093c3
<i>Htr1a</i>	GGATGTTTCTGTCCTGGT	CACAAGGCCTTCCAGAACT	59°C	Ref.4
<i>Htr2a</i>	AGAACCCCATTCACCATAGC	ATCCTGTAGCCCCAAGACTG	59°C	Ref. 4
<i>Grin2b</i>	TGGCCCTCAGCCTCATCACC	CATCACGGATTGGCGCTCCT	60°C	Ref. 5
<i>Oxtr</i>	GCACGGGTCACTAGTGTCAA	AAGCTTCTTGGCGCATTG	59°C	This study
<i>Oxt</i>	CACCTACAGCGGATCTCAGAC	CCGAGGTCAAGGCCAGTAAG	60°C	This study
<i>β-actin</i>	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT	59°C	6671509a1

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